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Gigantism: its induction by growth hormone in the skeleton of intact and hypophysectomized rats, and its failure following thyroidectomy [1]

by

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With 18 text figures

INTRODUCTION

The anterior lobe of the hypophysis regulates skeletal development in rats both through the secretion of its growth (or " somatotropic ") hormone and through its control of activity of the thyroid gland (Simpson, Asling and Evans, 1950). The main features of the interrelationships between growth hormone and thyroid hormone have been established by experiments in which young, actively growing rats were deprived of the pituitary gland, or of the thyroid gland, or of both, and received replacement therapy with growth hormone, or thyroid hormone, or both. Under such circumstances it has been demonstrated that

(1) growth hormone can maintain or restore active skeletal growth (as judged by increase in bone length and diameter);

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(2) thyroxine can maintain or restore skeletal maturation (as judged by appearance of secondary ossification centers and, later, by epiphyseal fusions and other synostoses);

(3) thyroxine, given with growth hormone, augments the growth-promoting action of the latter;

(4) the only circumstance under which thyroxine alone could promote vigorous and continued skeletal growth was when it was given to thyroidectomized animals, in whom the action is attributable to the secretion of endogenous growth hormone;

(5) the skeletal growth-stimulating ability of the growth hormone is exerted not only by enhancement of endochondral osteogenesis, with the intermediation of chondrogenesis, but also directly on bone formation itself, as may be shown by the activation of periosteal and intramembranous osteogenesis.

The majority of the experiments supporting these conclusions have been cited by Simpson et al. (1950). To achieve the critical basic conditions of maximum retardation of skeletal growth and maturation, it was necessary to perform the endocrine ablations as early in life as possible, usually at ages varying from birth to four weeks. To achieve results detectable by gross examination it was sometimes necessary to give the hormones for approximately a month, usually terminating the experiment at two months of age.

In animals treated at older ages, and for longer periods of time, the most important findings have been that growth hormone can induce gigantism in both body weight and skeletal dimensions, whether in intact or hypophysectomized adults (Evans et al., 1949) and that it can induce other skeletal changes corresponding to human acromegaly (Asling et al., 1954). This possibility of inducing gigantism in adults depends chiefly on a special characteristic of the skeleton of rodents, " lapsed union ", in which many epiphyseal ossification centers of the axial skeleton and long bones normally retain their cartilage plates until old age (Dawson, 1925, 1929). In hypophysectomized rats, even more of such lapsed unions may be present, due to the maturation-retarding influence of this endocrine deprivation. The number and location of these additional lapsed unions depends on the age at which hypophysectomy was

performed. The degree of " proportionality " in the gigantism induced by growth hormone may thus vary between intact and hypophysectomized animals, being affected by the number of ossification centers available for stimulation. Also, under specified circumstances, chronic administration of growth hormone to adult rats may lead to arthropathies, overgrowth or exostoses at certain bony prominences, and ectopic ossification in tendons and periarticular connective tissues, giving a condition corresponding to acromegaly in human beings.

There remain some unresolved fundamental problems in defining the interrelationships between growth hormone and thyroid hormone. In part these problems result from marked differences in the responsiveness of intact, hypophysectomized, and thyroidectomized rats to growth hormone. Furthermore, there are difficulties in evaluating the biological importance of potential contaminants in growth hormone extracts, even in minute amounts, when the hormone is given in large doses over a prolonged period of time. The fact that responsiveness to growth hormone is least in thyroidectomized rats suggests that the thyroidal augmentation of growth hormone, mentioned above, may have an importance not usually emphasized. In contrast, hypophysectomized rats are most responsive, but chronic experiments with such animals have not resolved the problem, perhaps due to a low level of thyroid activity (whether inherent or supported by minute contaminating traces of thyrotropic hormone in the growth hormone, undetectable in short-term assay tests). The unresolved problem is exemplified by the skeletal maturation which took place, although slowly, during chronic treatment of hypophysectomized rats with growth hormone. In them, epiphyseal unions occurred at sites in which such maturation was achieved in acute tests only by treatment with crystalline thyroxine (*e.g.*, Asling et al., 1949).

Although growth hormone treatment of thyroidectomized rats should answer these problems, this has not been possible in short-term treatment of young animals. A small amount of residual skeletal growth and maturation is observed in rats after thyroidectomy at an early age, which makes difficult the evaluation of the further effects obtained by growth hormone administration. It is desirable, therefore, that the definitive experiments be performed on adult thyroidectomized animals in which residual growth and maturation

is not a complication. The present study compares the effects of chronic injection of growth hormone, in high dosages, on the skeletal growth of adult intact, hypophysectomized, and thyroidectomized rats.

EXPERIMENTAL PROCEDURES

Similar circumstances prevailed in the experimental conditions in the three basic groups of animals, intact, hypophysectomized, and thyroidectomized (and corresponding controls). All were female rats of the Long-Evans strain, and had reached the growth plateau in body weight when selected for the experiments. All were fed on an optimal diet of natural foodstuffs. All received highly purified pituitary growth hormone, homogeneous by physicochemical tests. The hormone was administered intraperitoneally, six times weekly, for eight months or more, in dosages adequate to maintain vigorous gain in body weight.[1]

In the groups of thyroidectomized rats, thyroid destruction was accomplished by administration of radioiodine (I^{131}, 750 μc), a procedure chosen to give assurance that any aberrant thyroid tissue would also be destroyed (Asling et al., 1957). After a period of twelve weeks to allow for radioactive decay and elimination of any remaining I^{131}, each animal received a further, tracer dose of I^{131}, and the neck region was scanned by a scintillation crystal probe. Any animal which showed iodine concentration above the non-specific background observed over muscle was rejected on the presumption that active thyroid remnants persisted. On this basis, approximately half of the original group was rejected. A repetition of the I^{131} tracer injection was made at the termination of the experiment. The neck region was then dissected, and the

[1] The growth hormone administered to the intact and hypophysectomized rats in these experiments was prepared by C. H. Li, according to the method described (Li et al., 1945). That administered to the thyroidectomized rats was prepared by Stanley Ellis, according to the method described (Ellis et al., 1954). The authors acknowledge gratefully the generous amounts of highly purified hormone which have been used.

Since the intact and hypophysectomized rats have been the subject of reports on tumors induced by growth hormone, details of their experimental conditions have already been described (Moon et al., 1950, 1951). In addition, these animals provided the basis for a report on experimentally induced acromegalic osteoarthropathies (Asling et al., 1954).

TABLE 1.

Experimental Conditions for the Chronic Injection of Growth Hormone in Adult Female Rats.

Group	No. of Rats	Age (Weeks)			Growth Hormone		Body Weight Gain		Body Length
		Onset of Deficiency	Onset of Injections	Termination	Duration Weeks	Dosage mg/day	gms total	gms/week	mm
Intact Controls	11	—	—	103	—	—	51	0.7	414
Intact+ Growth Hormone	7	—	34	103	69	0.4–3.0	277	4.0	454
Hypophysectomized Controls	12	28	—	86	—	—	−2	0.0	373
Hypophysectomized +Growth Hormone	8	28	30	86	56	0.05–2.5	255	6.0	452
Normal Controls	11	—	—	81	—	—	49	1.4	413
Thyroidectomized Controls	12	33	—	81	—	—	37	0.5	391
Thyroidectomized +Growth Hormone	12	33	45	81	36	0.5–5.0	197	5.5	410

negligible uptake of iodine by paratracheal tissue was confirmed (Addendum, Table I).

The experimental conditions in the three groups of treated rats are summarized in Table 1.

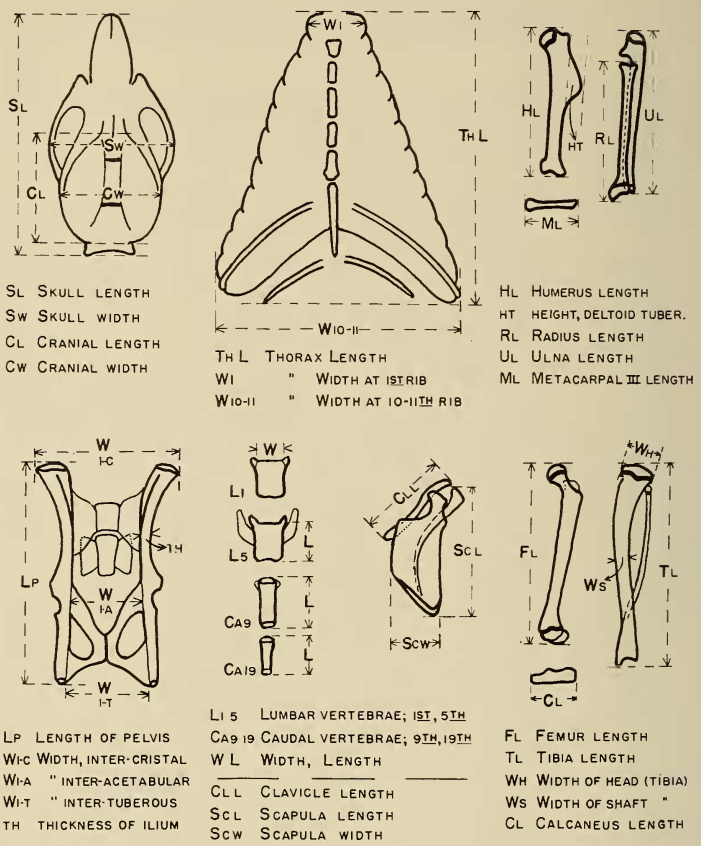

FIG. 1.

Diagrams illustrating landmarks used in making skeletal measurements on roentgenograms. Heavy bars in epiphyses of long bones indicate sites of epiphyseal plates persisting in adults; similar synchondroses are shown in skull base, ilium, ischium, and caudal vertebrae.

In one of the groups (thyroidectomized rats treated with growth hormone) after 36 weeks of injections, half of the animals (and of their controls) were sacrificed for gross and histological examination. The remaining half were maintained on the same dose of

growth hormone, but for four more weeks they received thyroxine injections also (5 μg/day).

At the time of autopsy of all animals, whole-body roentgenograms were made on fine-grain film, using a target-to-film distance of one meter to avoid distortion due to projection. Selected bones were fixed for histological study of osteogenesis.

Measurement of lengths and other dimensions of individual bones or of complexes of bones, were made from the roentgenograms. The dimensions selected for measurement include representatives of both axial and appendicular skeleton, and are shown in the diagrams in figure 1. Among them are (a) bones which retain one or two epiphyseal cartilage plates and whose increase in length would result from endochondral osteogenesis (*e.g.*, length of ulna, tibia, pelvis), (b) bones which increase in length by endochondral osteogenesis but in which epiphyseal fusion was complete before the onset of the experiment (*e.g.*, metacarpal, calcaneus), (c) bony dimensions which would increase only by periosteal osteogenesis (*e.g.*, width of tibial shaft, height of deltoid tuberosity), and (d) bones or groups of bones which increase in size by a combination of endochondral and periosteal or intramembranous osteogenesis (*e.g.*, the skull). The measurements were made under 7.5 × magnification, with vernier calipers reading to 0.1 mm, with an error of measurement lying between one and two parts per hundred. The majority of measurements on roentgenograms indicate the extent of growth dependably, but they agree with the actual length of the bone only if there has been no foreshortening of the image by angulation. In the tibia, for example, the mean length of the roentgenographic image in 22 normal rats in this experiment was 40.5 ± 0.16 mm, and of the dissected bone, 40.3 ± 0.17 mm. In bones like the humerus and femur, moderate foreshortening of the image occurs by angulation but discrepancies from actual length are relatively constant. In bones such as the scapula, minor differences in the animal's position may result in major changes in bony angulation and image foreshortening, and therefore only the extremes of response may be judged.

RESULTS

Body Weight Gain

The growth in body weight, which was summarized in table 1, is shown in detail in figures 2 to 4. Normal controls (figure 2, figure 4) made the slight weight gains usually seen in plateaued female rats; the hypophysectomized and thyroidectomized controls (figure 3, figure 4) lost weight after the operation but subsequently regained or even slightly surpassed the initial level.

All growth hormone injected rats showed essentially similar and substantial gains in body weight, the groups doubling their initial weight as shown by figures 2 to 4. Late in the prolonged experimental period increasing difficulty was experienced in maintaining the hypophysectomized rats, and the average weight of the group

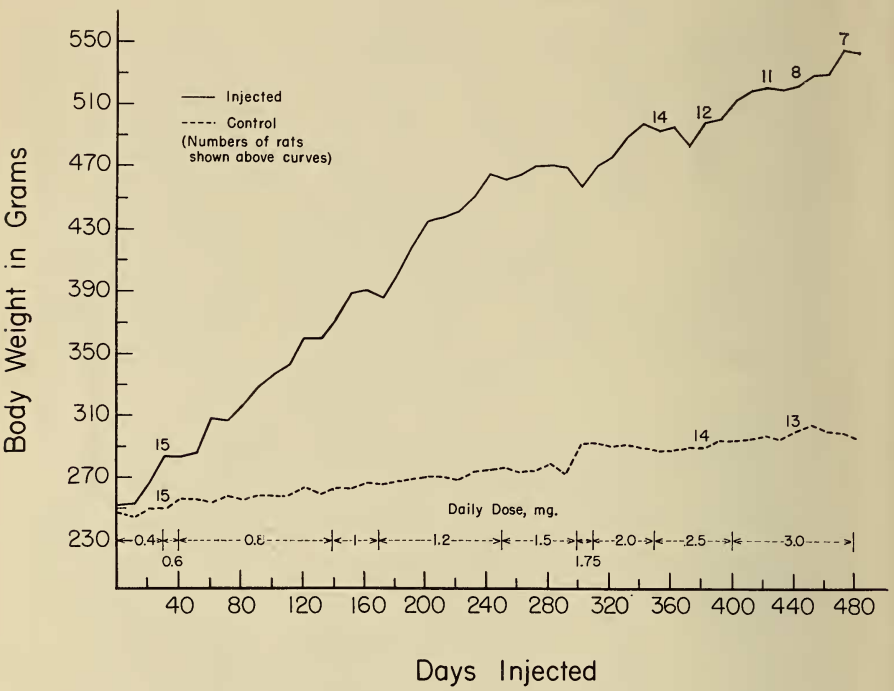

Fig. 2.

Body weight of intact adult female rats, injected for 485 days with pituitary growth hormone (from Moon et al., 1950).

declined; the weight gain given for the group in Table 1 is for the preceding 43-week period of active weight gain. The thyroidectomized rats receiving growth hormone continued to gain weight throughout at the maximum rate. In spite of the addition of thyroxine at the end of the experiment to part of the group, the change in rate was not statistically significant (29 $\pm$ 4.2 grams during the 28-day period of treatment with growth hormone and thyroxine, versus 22 $\pm$ 8.6 grams for the same animals during the preceding 28 days with growth hormone alone).

Weights of Viscera

The weights of representative endocrine and non-endocrine organs in the growth-hormone-treated intact and hypophysectomized rats have been reported previously (Evans et al., 1948;

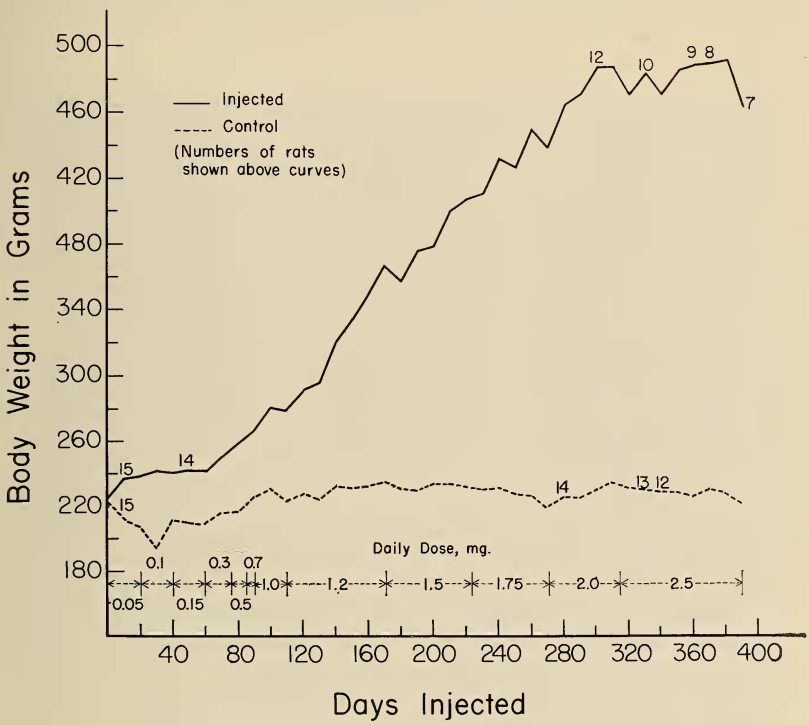

Fig. 3.

Body weight of hypophysectomized adult female rats, injected for 392 days with pituitary growth hormone (from Moon et al., 1951).

Moon et al., 1951). Such measurements were also made on the growth-hormone-treated thyroidectomized rats. These data are presented in the Addendum (Table I).

Body Length.

The body length increase of intact, hypophysectomized, and thyroidectomized rats, under influence of growth hormone, differed markedly (Table 1). The intact treated rats were 10% longer than

normal, corresponding closely to results previously reported (Evans et al., 1948). The lengths of the hypophysectomized treated rats do not represent their actual growth. The majority of these animals showed arthropathies which, in the vertebral column resulted in kyphosis and prevented straightening the animal, even under anesthesia, to allow correct measurement of the length achieved.

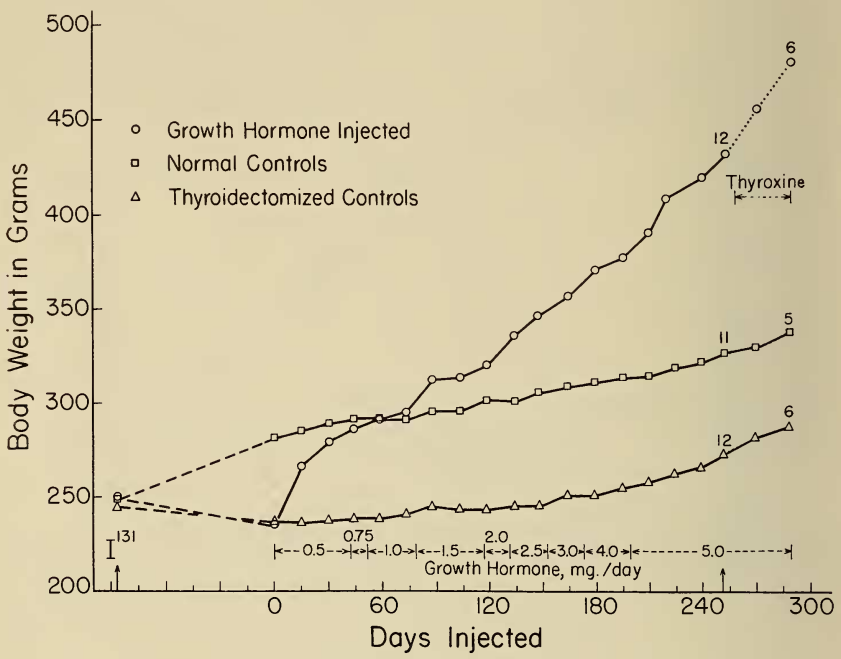

Fig. 4.

Body weight of thyroidectomized adult female rats, injected with pituitary growth hormone. After 251 days part of the rats in each group were sacrificed; the remainder continued to receive growth hormone with thyroxine supplement (5 μg/day) for 28 days.

On the basis of tail length, which could be measured accurately, these animals were 7% longer than intact growth-hormone-treated rats, and thus showed the most marked growth of any of the groups. As will be seen, this impression was confirmed by measurements of individual bones.

The thyroidectomized rats injected with growth hormone showed a paradoxical growth response. In spite of doubling of body

weight, their growth in length scarcely exceeded that of thyroidectomized controls, and did not surpass normal adults. This lack of correspondence between weight and length is shown clearly in photographs of representative animals. In figure 5, while the thyroidectomized treated rat on the right shows a greater mass than the normal control on the left, body lengths are the same.

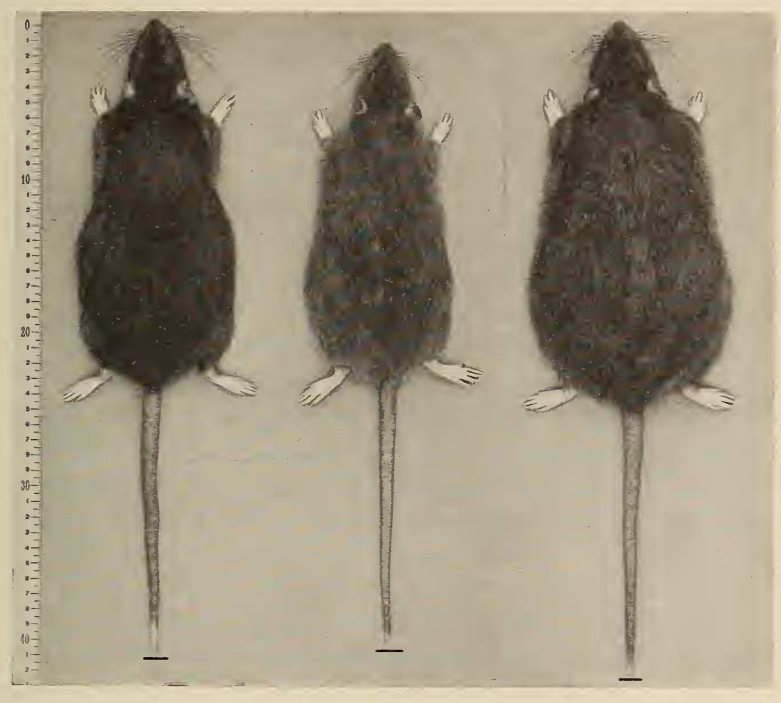

Fig. 5.

Photographs of representative rats from the groups whose body weights are shown in figure 4. On left; normal control; in center, thyroidectomized control; on right, thyroidectomized rat treated with growth hormone. The scale is numbered in centimeters.

The greater mass is not due to accumulation of adipose tissue, as is demonstrated by measurements of specific gravity and muscle nitrogen content (Addendum, Table I). The specific gravity of normal rats (1.03, as determined by water displacement of the intact carcass) was unaltered by thyroidectomy or by growth-

hormone treatment. Adult female rats of weight and length comparable to those treated with growth-hormone, in whom the excess weight represented obesity due to hypothalamic injury, had a specific gravity of 0.98 (Van Dyke et al., 1957). In the gastrocnemius muscle (whose weight remained proportionate to body weight) the nitrogen content was only slightly lessened in thyroidectomized rats, with or without growth-hormone treatment. With the addition of thyroxine, muscle nitrogen content was normal.

Dimensions of Bones.

Measurements of individual bony dimensions (figure 1) also showed marked difference in the skeletal response to growth hormone in the three groups of animals. The complete protocol of these measurements is given in the Appendix, which provides the means and standard errors (Table A), the percent of increase over the appropriate control value (Table B), and the percent of increase above normal adult dimensions effected by growth hormone (Table C). From this comprehensive set of tables, representative bony dimensions have been selected for presentation here, in order to demonstrate more clearly the differences in response and the factors which affect them (Tables 2 and 3). In table 2, the actual dimensions are shown, while table 3 presents the skeletal growth in terms of gigantism (percent increase above normal). Furthermore, the various bony dimensions are classified (Table 3) according to whether their increase is effected by endochondral or periosteal osteogenesis. The endochondral group is further subdivided according to the presence or absence of epiphyseal cartilage plates at the beginning of the experiment (figure 1).

The following conclusions may be drawn from both actual and percentage increases in bony dimensions.

1. Thyroidectomized rats showed a unique response to administration of the growth hormone. There was negligible increase in body and bone length (endochondral osteogenesis) even though substantial increases in bony widths (periosteal osteogenesis) occurred. As a result, they showed an extreme lack of uniformity in the increase of their various bony dimensions. When thyroxine was added these animals showed increase in body and bone length.

TABLE 2.

Skeletal Dimensions in Intact, Hypophysectomized, and Thyroidectomized Adult Female Rats, Uninjected and Injected Chronically with Growth Hormone.

Group	Length			Width or Thickness		
	Body mm	Tibia mm	Metacarpal mm	Humerus, Delt Tub. mm	1st Lumb. Vertebra mm	Tibia Shaft mm
Intact Controls	414	40.3	7.6	2.3	6.0	2.8
Intact+Growth Hormone	454	44.2	7.6	2.7	6.5	3.4
Hypophysectomized Controls	372	38.3	7.5	2.0	5.9	2.7
Hypophysectomized+Growth Hormone	452	47.1	7.7	3.3	7.6	3.7
Thyroidectomized Controls	391	39.1	7.4	2.2	5.8	2.7
Thyroidectomized+Growth Hormone	410	40.6	7.6	3.1	6.7	3.1
Thyroidectomized+Growth Hormone+Thyroxine Supplement	421	41.6	7.5	3.2	6.7	3.1

TABLE 3.

Percent Above Normal in Skeletal Dimensions of Growth Hormone Injected Intact, Hypophysectomized, and Thyroidectomized Adult Female Rats.

Treatment	Length			Width or Thickness		
	Body	Tibia	Metacarpal	Humerus, Delt. Tub.	1st Lumb. Vertebra	Tibia Shaft
Intact+Growth Hormone	10	10	0	17	8	21
Hypophysectomized+Growth Hormone	9	17	0	44	27	32
Thyroidectomized+Growth Hormone	0	0	0	34	12	11
Thyroidectomized+Growth Hormone+Thyroxine Supplement	3	3	0	35	12	11
Hypophysectomized Controls	–10	–5	0	—	0	—
Thyroidectomized Controls	–5	–3	0	—	0	—
Mode of Growth	Epiphysis patent		Epiphysis fused	Periosteal Osteogenesis		
	Endochondral Osteogenesis					

2. In every bony dimension studied, the response to growth hormone was greatest in hypophysectomized rats. Intact animals showed somewhat lesser, but more nearly uniform increases.

3. In those bones in which epiphyseal union had occurred before the onset of the experiments (*e.g.*, metacarpals, calcaneus), growth hormone injections produced no bony elongation in any of the groups.

Histological Studies: Endochondral Osteogenesis.

The osteogenetic activity which led to these differences in dimensions was analyzed by histological studies of the proximal end of the tibia. Endochondral osteogenesis was examined in sagittal sections of the epiphysis (figures 6 to 12), and periosteal osteogenesis in cross sections of the shaft taken at the junction of the upper and middle thirds of the bone (figures 13 to 18).

The basic condition, that of normal controls at this advanced age, is illustrated in figure 6. The region of the proximal epiphyseal cartilage plate corresponded closely to that previously described for rats of this age and strain (Becks et al., 1945). On the epiphyseal side a virtually complete layer of bone sealed the cartilage plate from the epiphyseal marrow. On the diaphyseal side the plate was similarly sealed for the greater part of its length, as is generally characteristic for growth arrest in this region (Asling and Evans, 1956). A few slender strands of primary spongiosa, containing cores of cartilage matrix surrounded by a thin lamina of bone, extended into the marrow cavity and connected with sturdier bony trabeculae deeper in this cavity. Intermittently the sealing lamina of bone was interrupted, and a tuft of marrow was encroaching upon the cartilage plate. However, this slight erosion of the plate was balanced by some evidences of chondrogenesis, in the form of a few short columns or conical nests of proliferating flattened chondrocytes. Elsewhere the cartilage was composed of broad areas of noncellular matrix. In general, the histological appearance may be summarized as that of a cartilage plate at which endochondral ossification is proceeding at such an extremely slow rate that growth in bone length is negligible.

In both hypophysectomized and thyroidectomized rats, endochondral osteogenesis had completely ceased. The resulting histo-

logical appearance was identical in these two groups of animals (figures 8 and 10, respectively). The cartilage plate was entirely sealed from the diaphyseal marrow by a thin lamina of bone, and the marrow cavity itself was almost devoid of bone. In the cartilage plate there were somewhat greater numbers of conical clusters of flattened cells than were found in the intact controls. Elsewhere, the noncellular matrix was almost as abundant as seen in the normal controls. Toward the diaphyseal side of the plate, some chondrocytes were rounded rather than flattened, and lay in slightly enlarged lacunae; tiny osseous masses extended from the sealing lamina into spaces formerly occupied by the most distal row of such enlarged lacunae.

As a result of the administration of growth hormone to adult intact animals all essential characteristics of endochondral osteogenesis were in progress, even after so long a period of time (figure 7). The plate was slightly wider (table 4) and the number of columns and clusters of cartilage cells was increased. Toward the diaphysis, these cell groups often showed moderate hypertrophy and rounding of chondrocytes, and at the marrow junction their enlarged lacunae were undergoing erosion. Delicate short bony trabeculae were connected to the cartilage between every second or third cell cluster; deeper in the marrow cavity these bony elements were remodelled and reorganized into a sturdier secondary spongiosa.

The administration of growth hormone to adult hypophysectomized animals stimulated marked endochondral osteogenesis and widened the cartilage plate (figure 9, table 4). Many columns of flattened proliferating chondrocytes extended through the width of the plate, and terminated in a zone of hypertrophic rounded cells two to five cells deep. The enlarged lacunae of the latter were subject to active erosion by marrow tufts, and bony replacement and subsequent remodelling was similarly active. In fact, the appearance corresponded closely with that seen in young actively growing rats approximately 100 days of age (Becks et al., 1945). This growth activity, sustained in adults for a prolonged period, accounts for the fact that the gigantism achieved by hypophysectomized rats receiving growth hormone exceeded that found in any of the other treated groups.

When adult thyroidectomized rats received growth hormone the epiphyseal cartilage plate showed abnormalities not

previously encountered (figure 11). In spite of marked widening of this plate (table 4), and the presence of some long columns of chondrocytes, there were no evidences of effective endochondral osteogenesis. The greater part of the area of the plate was occupied by large islands of noncellular, degenerated matrix. Other areas showed degeneration of cells, empty lacunae, and changes in the matrix suggesting that still more islands of degeneration were being formed. A few tufts of marrow elements had encroached on the cell clusters and created the impression of irregular erosion. Rarely, one of the islands of degenerated cartilage had been bypassed by erosion (*e.g.*, at the far right of figure 11). However, in the main the diaphyseal aspect of the cartilage plate was sealed from the marrow by arches of bone whose bases continued into long pillars of old, reorganized trabeculae. The inactivity and grotesque distortion of cartilage structure seen in this plate accounted adequately for the failure of longitudinal growth of the bone by endochondral osteogenesis, which was described earlier on the basis of gross measurements.[1]

When thyroxine was added to the growth hormone therapy of adult thyroidectomized rats late in the experiment, reactivation of endochondral osteogenesis resulted (figure 12). The histological appearance, both of the cartilage plate and of the adjacent bony trabeculae, was almost identical with that seen with growth hormone treatment of the adult hypophysectomized rats (figure 9). The effectiveness of this activity in producing actual growth in bone length was demonstrated by the residue of degenerated islands of cartilage which now lay deeper in the diaphyseal marrow cavity. They remained unresorbed, became invested with bone, and formed a marker for the former site of the cartilage plate, before the period of thyroxine supplementation.

Periosteal Osteogenesis.

Enhancement of osteogenic activity *per se*, resulting from direct stimulation of osteoblasts (as distinguished from osteogenesis in

[1] It will be remembered that the growth hormone-treated thyroidectomized rats exceeded their controls in bone length very slightly, although not exceeding normal, and this scanty activity may be reflected in the irregular line of erosion of the plate. It is important to notice that growth hormone did exert some effect on this cartilage plate, even though that effect was abnormal and could not result in true endochondral osteogenesis and growth.

TABLE 4.

Histologic Measurements on the Tibias of Intact, Hypophysectomized, and Thyroidectomized Adult Female Rats, Uninjected and Injected Chronically with Growth Hormone.

Group	No. of Rats	Proximal Epiphyseal Cartilage Plate Width, micra	Cross Section of Shaft Bone Area, mm²
Intact Controls	22	136 ± 3.5	3.23 ± 0.06
Intact+Growth Hormone	8	153 ± 5.0	4.05 ± 0.11
Hypophysectomized Controls	9	152 ± 4.5	2.80 ± 0.12
Hypophysectomized+Growth Hormone	8	247 ± 13.6	4.90 ± 0.48
Thyroidectomized Controls	12	134 ± 3.9	2.90 ± 0.08
Thyroidectomized+Growth Hormone	6	240 ± 14.3	4.38 ± 0.33
Thyroidectomized+Growth Hormone+Thyroxine Supplement	6	193 ± 12.0	4.58 ± 0.27

which enhancement of endochondral activity is prerequisite) could be demonstrated by examination of periosteal osteogenesis in sections of the diaphysis of the tibia. All animals receiving growth hormone, whether they were intact, hypophysectomized, or thyroidectomized, showed this type of osteogenesis. Figures 13, 14, and 15 show sections from untreated adult intact, hypophysectomized, and thyroidectomized rats, respectively; their variation in structure was insignificant. The sections from corresponding treated animals are shown in figures 16, 17, and 18. All had grown, and the additional bony mass was demonstrable by planimetric measurement of the area of bone in these cross-sections (table 4). The incremental lines seen in each section from a treated animal show the outline of the bone before growth hormone treatment started, and show that they correspond closely to the untreated controls. It is noteworthy that the bone added under the influence of growth hormone was deposited unequally, *i.e.*, not in a circumferential "tree-ring" pattern. The greatest amount was on the medial (subcutaneous) surface of the tibia, extending around the medial border to the medial half of the posterior surface. The remaining surfaces showed little accretion, although the borders between these surfaces showed increased prominence. This regional pattern of apposition was seen, with minor variations, in all treatment groups, and indicated that regional selective factors were more important than differences in endocrine status in determining local response to growth hormone stimulus.

Arthropathies.

Inasmuch as reference has been made to the growth-hormone-induced bone and joint changes which have previously been reported for the intact and hypophysectomized rats described here (Asling et al., 1954), it should be mentioned that joint deformities and ossification in ligaments and tendons were negligible or absent in the thyroidectomized animals treated with growth hormone, although exostoses and bony thickening occurred.

DISCUSSION

These experiments have reaffirmed observations that growth hormone can re-establish growth in adult female rats (intact or

hypophysectomized) in which a growth plateau had been reached. Body weight, body length, and the dimensions of individual bones all were increased above normal (gigantism). In thyroidectomized rats, however, although body weight (including muscle mass and visceral weight) showed a corresponding increase, the skeletal response did not follow the same pattern. Bone formation as such (including periosteal and endosteal osteogenesis) was stimulated, and the thickness of bones was increased, but endochondral " osteogenesis " was unresponsive, and the bones did not lengthen. Thus the growth hormone stimulated osteoblastic function in the absence of the pituitary or the thyroid gland, as well as in intact animals, but the chondrogenetic activity at epiphyseal plates which would lead to bony lengthening was unresponsive in the absence of the thyroid gland.

Scow (1959) has analyzed critically the growth response of thyroidectomized-hypophysectomized rats to growth hormone and/or thyroxine, giving special attention to the weights of the non-endocrine organ systems as well as to the protein anabolism which contributes to the mass of the skin, the muscles, the skeleton, and the chief viscera of the torso. He reported that although some of these phenomena are responsive to growth hormone alone, others require the support of thyroxine supplementation in order to show significant increase. He concluded that in some instances the amount of thyroid hormone required is very small, and repeated his earlier suggestion (Scow et al., 1949) that it may even be met by the slight residual activity of the thyroid gland in hypophysectomized rats.

The present experiment raises questions corresponding to those posed by Scow. In placing the growth mechanisms under the strain of overstimulation to the point of gigantism it has allowed further distinctions to be made concerning the hormonal requirements of the histogenetic mechanisms responsible for skeletal growth.[1] The failure of growth hormone to induce endochondral osteogenesis in the thyroidectomized rats, and the attending grotesque

[1] We wish to acknowledge that approximately ten years ago Scow, in a personal communication to one of the authors (H.M.E.), proposed that an effort be made to accelerate the growth of thyroidectomized rats beyond the normal rate, and predicted that in the absence of thyroid hormone growth hormone would be inadequate to achieve this acceleration. The present experiment, although differing in design from his proposal, was substantially inspired

alteration of the epiphyseal cartilage plate's histological structure (figure 11) indicate that the essential conditions for activation of the chondrogenetic phase of osteogenesis were not met until thyroxine supplementation was given (figure 12). In the intact rats which became gigantic under treatment with growth hormone a functioning thyroid gland was present. In the hypophysectomized rats of this experiment, which showed most marked response to growth hormone, a thyroid gland was also present, but its functional importance is not clear. On the one hand, it has long been held that the deprivation of thyrotropic hormone which follows hypophysectomy renders the thyroid gland functionally insignificant. On the other hand, there is gathering biochemical evidence, obtained by highly sensitive radio-chromatographic procedures, that hypophysectomized rats do synthesize and release thyroid hormone, even though slowly and at low levels (Taurog et al., 1960). It has also been demonstrated that the skeletal response to thyroxine is one of the most sensitive of this hormone's actions, and that lower doses than were formerly thought significant may stimulate appreciable activity. For example, 0.25 μg/day of l-thyroxine were found adequate to maintain a near-normal rate of all phases of skeletal morphogenesis in completely thyroidectomized rats for a period of three months (Asling and Evans, 1963). The same dosage served so to sensitize hypophysectomized rats to the effects of growth hormone that the sensitivity of the tibial cartilage assay procedure was increased several-fold (Geschwind and Li, 1955). The lower limits of the thyroxine dose-sensitivity of the skeleton have not been established and especially the lower limit of this hormone's capacity to augment the action of growth hormone.

Although this experiment does not exclude the possibility of thyrotropic hormonal contaminant in the growth hormone administered to the hypophysectomized rats, other studies have shown that this is insignificant in chronic experiments. Evans et al. (1958) demonstrated that a sustained action of growth hormone in hypophysectomized rats (in their study, calorigenesis) could not

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by his suggestion. It verifies his prediction from the standpoint of endochondral osteogenesis and growth in body length, although yielding other conclusions from the standpoint of osteoblastic activity *per se* (*e.g.*, periosteal osteogenesis) and growth in body weight.

be attributed to contaminating thyrotropic hormone, for the thyroids of the treated animals became refractory to thyrotropic hormone (as judged by both functional and histological tests) and regressed to hypophysectomized control levels within 50 days of treatment. (Active endochondral osteogenesis was still demonstrable in hypophysectomized rats in the present experiment after 392 days of growth hormone injections.) In acute experiments in which growth hormone was intentionally contaminated with thyrotropic hormone, Geschwind and Li (1955) showed that the level of thyrotropic hormone must be extremely high to produce significant augmentation. However, even the question of contamination does not invalidate the conclusion that the essential condition which allowed growth hormone to stimulate vigorous endochondral osteogenesis was the presence of the thyroid gland or (in thyroidectomized rats) replacement therapy with its hormone.

In considering differential effects on cellular mechanisms of bone growth, it has long been known that the deeper layers of periosteum contain cells of osteogenic potentiality. In fractures, their role in the formation of callus and bone is well established (McLean and Urist, 1961). Simpson et al. (1953) have shown their responsiveness to growth hormone in regeneration of the calvarium. The present studies indicate that this hormone is the adequate endocrine stimulus for their activation. However, in activation of the epiphyseal cartilage plate, the significance of the equivalent cells (*i.e.*, those of chondrogenic potentiality) has received inadequate attention until recently. It has been customary to direct the most attention to the long columns of flattened proliferating chondrocytes which are so conspicuous in the epiphyseal cartilage plate of actively growing animals, whether the growth be that characteristic for youth or that induced by growth hormone in hypophysectomized animals. In fact, the tibia line assay procedure for growth hormone is based on the widening of the cartilage plate which results from proliferation and hypertrophy in these cell columns (Evans et al., 1943; Greenspan et al., 1949). On the other hand, scanty notice has been given to the progenitors of these cells, which lie immediately next to the bone of the epiphysis, in a narrow " germinal zone ", also known variably as the zone of reserve cartilage cells, the embryonic zone, the zone of undifferentiated cells, or, in very young animals, the anlage cartilage. Proli-

feration among these cells is inconspicuous. However, their importance has recently been emphasized by the experiments of Rigal (1962, 1964), using procedures more sensitive than conventional histological study. In that work, organ cultures were made of slices through the epiphyses of rabbits, tritiated thymidine being added to the culture medium. By examining autoradiographs of sections of these explants, proliferative potential was demonstrated in the germinal zone of the epiphyseal cartilage plate, and it was established that these cells were the progenitors of chondrocytes in the zone of flattened proliferating cells. When the explants were taken from growth hormone treated rabbits, the frequence of labelling in the germinal zone was increased five- to ten-fold, although labelling in the subjacent columns was only slightly increased.[1]

Although the sensitive procedures employed by Rigal were not used in the present study, clusters of chondrocytes in the germinal zone were more easily identified in sections from actively growing epiphyses (*e.g.*, figures 9 and 12) than in those from the other groups of animals. Procedures employing the thymidine-H_3 labelling technique are necessary to establish whether these cells depend on thyroid hormonal support of growth hormone for their activation.

SUMMARY

Anterior hypophyseal growth hormone was administered in high dosages and for prolonged periods of time (eight months or longer) to adult intact, hypophysectomized, and thyroidectomized rats, and comparisons were made of their skeletal growth by roentgenographic and histologic procedures.

The most striking result was the unique response of the thyroidectomized rats to growth hormone. They were unable to exceed normal body length, although doubling of body weight took place, as in the other treated groups. Endochondral osteogenesis was at best only slightly enhanced over control levels, and even with

[1] In another phase of these studies, Rigal showed that tibial epiphyseal explants taken from animals which had not been pre-treated with growth hormone did not respond to growth hormone which was added to their culture medium.

APPENDIX

TABLE A.

Measurements of the Skeleton of Intact, Hypophysectomized and Thyroidectomized Adult Female Rats, Uninjected and Injected Chronically with Growth Hormone.

Number of Rats	Normal		Hypophysectomized		Thyroidectomized		Injected, Thyroxine Supplement
	Uninjected 22	Injected 8	Uninjected 9	Injected 8	Uninjected 12	Injected 6	6
	mm	mm	mm	mm	mm	mm	mm
AXIAL SKELETON							
Body Length							
Total	414 ±1.57	454 ±4.7	373 ±3.6	452 ±12.7	391 ±2.78	410 ±3.66	421 ±3.83
Nose-Anus	224 ±1.13	250 ±2.8	199 ±2.4	233 ±5.6	210 ±2.03	221 ±1.01	234 ±2.02
Anus-Tail Tip	189 ±1.38	204 ±2.3	173 ±1.8	218 ±9.1	182 ±1.88	189 ±4.06	187 ±2.37
Skull							
Length	47.2 ±0.25	50.6 ±0.40	42.8 ±0.66 [1]	49.0 ±1.03	45.6 ±0.25	46.3 ±0.42	47.9 ±0.40
Width	23.4 ±0.15	27.0 ±0.24	22.1 ±0.21 [1]	26.7 ±0.50	23.0 ±0.21	25.5 ±0.56	26.9 ±0.35
Cranium							
Length	21.4 ±0.11	23.6 ±0.33	21.3 ±0.24 [1]	25.2 ±0.41	20.9 ±0.16	21.0 ±0.37	22.3 ±0.16
Width	15.6 ±0.13	16.8 ±0.24	15.5 ±0.28 [1]	18.8 ±0.52	15.5 ±0.09	17.6 ±0.37	17.3 ±0.36
Thorax							
Length	56.4 ±0.44	65.4 ±0.63	57.6 ±0.76	69.5 ±3.19	52.2 ±0.86	57.1 ±0.67	59.3 ±0.76
Width (level of lst rib)	13.2 ±0.09	14.6 ±0.20	12.7 ±0.20	15.9 ±0.48	13.2 ±0.25	14.3 ±0.25 [1]	14.4 ±0.16
Width (level of 10th-11th rib)	53.5 ±0.68	64.1 ±1.27	[3]	[3]	51.0 ±0.96	56.3 ±1.02 [1]	58.5 ±1.78
Vertebrae							
Length, 5th Lumbar	7.7 ±0.08	9.0 ±0.15	7.2 ±0.18	8.9 ±0.50	7.2 ±0.07	7.5 ±0.11	8.0 ±0.05
Length, 9th caudal	9.8 ±0.05	11.1 ±0.13	9.3 ±0.09	11.9 ±0.36	9.4 ±0.09	10.1 ±0.33	10.0 ±0.13
Length, 19th caudal	6.8 ±0.06	7.7 ±0.12	6.5 ±0.09	7.9 ±0.24	6.8 ±0.08	6.9 ±0.13	7.0 ±0.11
Width, 1st Lumbar	6.0 ±0.05	6.5 ±0.16	5.9 ±0.05	7.6 ±0.27	5.8 ±0.10	6.7 ±0.12	6.7 ±0.06

APPENDICULAR SKELETON							
Pectoral girdle							
Scapula, length	24.2 ±0.19	27.2 ±0.67	25.8 ±0.97	32.8 ±0.94	24.4 ±0.27	24.9 ±0.62[1]	25.9 ±0.31
Scapula, width	9.7 ±0.20	11.8 ±0.31	9.0 ±0.28	12.9 ±0.57	9.1 ±0.24	10.9 ±0.44[1]	10.8 ±0.23
Clavicle, length	15.7 ±0.14	17.4 ±0.75	13.0 ±0.20	15.6 ±0.92	14.8 ±0.20	16.4 ±0.39[1]	16.7 ±0.26
Fore-limb							
Humerus, length	29.0 ±0.12	32.8 ±0.41	26.2 ±0.43	32.2 ±0.79	27.1 ±0.18	28.8 ±0.29[1]	30.1 ±0.36
Humerus, width of deltoid tuberosity	2.3 ±0.06	2.7 ±0.12	2.0 ±0.10	3.3 ±0.21	2.2 ±0.06	3.1 ±0.13[1]	3.2 ±0.11
Radius, length	25.8 ±0.17	29.1 ±0.40	24.2 ±0.27	29.6 ±0.70	25.0 ±0.15	25.9 ±0.24[1]	26.9 ±0.26
Ulna, length	32.8 ±0.15	36.2 ±0.20	31.1 ±0.27	37.6 ±0.88	31.8 ±0.19	32.9 ±0.20[1]	33.5 ±0.35
Metacarpal, length	7.6 ±0.03	7.6 ±0.08	7.5 ±0.12	7.7 ±0.05	7.4 ±0.07	7.6 ±0.15[1]	7.5 ±0.09
Pelvic girdle							
Length	42.9 ±0.29	48.7 ±0.33	40.6 ±0.59	50.9 ±1.73	41.0 ±0.50	43.1 ±0.44	44.4 ±0.46
Width, intercristal	27.5 ±0.30	34.3 ±0.97	23.6 ±0.52[2]	35.0 ±3.50[2]	26.0 ±0.32	29.7 ±0.91	32.6 ±0.76
Width, interacetabular	13.6 ±0.21	16.4 ±0.40	11.8 ±0.33[2]	17.6 ±1.49[2]	12.9 ±0.16	14.8 ±0.25	15.9 ±0.16
Width, intertuberous	14.9 ±0.31	17.5 ±0.56	13.6 ±0.14[2]	17.6 ±0.82[2]	14.6 ±0.32	17.0 ±0.22	17.5 ±0.20
Thickness of bone, ilium	2.2 ±0.04	2.7 ±0.10	1.9 ±0.01	3.1 ±0.01	2.1 ±0.03	2.7 ±0.12	2.8 ±0.08
Hind-limb							
Femur, length	34.4 ±0.18	38.6 ±0.40	33.1 ±0.51	42.5 ±1.17	33.0 ±0.32	34.9 ±0.30	35.6 ±0.45
Tibia, length	40.3 ±0.17	44.2 ±0.38	38.3 ±0.39	47.1 ±1.10	39.1 ±0.33	40.6 ±0.44	41.6 ±0.53
Tibia, width of head ant.-post.	6.9 ±0.04	7.6 ±0.09	6.9 ±0.13	8.0 ±0.20	6.7 ±0.08	7.1 ±0.19	7.4 ±0.11
Tibia, width of shaft, lateral	2.8 ±0.03	3.4 ±0.10	2.7 ±0.08	3.7 ±0.10	2.7 ±0.04	3.1 ±0.11	3.1 ±0.06
Calcaneus, length	9.1 ±0.06	9.1 ±0.10	9.2 ±0.11	9.2 ±0.14	8.9 ±0.08	9.3 ±0.22	9.3 ±0.27

[1] Mean based on 1 animal less than shown.
[2] Mean based on 4 animals less than shown.
[3] Not measurable with accuracy on roentgenograms.

massive doses of hormone they only maintained normal lengths of body and bones. However, vigorous growth by periosteal osteogenesis took place, and individual bones were much thicker than normal. When thyroxine supplementation was added the thyroidectomized animals resumed effective endochondral osteogenesis, with increased length of body and bones, and corresponding histological activity.

The skeletal response to growth hormone was greatest in hypophysectomized rats and least in thyroidectomized rats, the response of intact animals being intermediate. In those bones in which epiphyseal union has occurred before the onset of the experiments, growth hormone injections produced no bony elongation in any of the three groups of rats.

It is concluded that generalized bony overgrowth (skeletal gigantism) was not produced by growth hormone alone, but required the additional support of the thyroid hormone.

APPENDIX

Tables A to C present detailed analyses of some dimensions of the skeleton in intact, hypophysectomized, and thyroidectomized rats, untreated and treated with growth hormone. Table A contains means and standard errors of actual measurements, the majority of which were made on roentgenograms; its arrangement and the dimensions measured correspond exactly to those reported in a previous study on proportionality (Evans et al., 1949). In tables B and C, which give percentages of stunting following endocrine ablation and of increase induced by growth hormone, the bony dimensions are regrouped to show those which are primarily endochondral in their mode of growth, those which are more complex (usually both endochondral and periosteal), and finally those which are chiefly by periosteal osteogenesis. In these tables numerical values are given only when statistically significant. In the majority of instances these represent a " p " value equal to or less than 0.01, but in five cases this lay between 0.02 and 0.01 (length of 5th lumbar vertebra and of femur in hypophysectomized controls; interacetabular width of pelvis in thyroidectomized controls; width of the head of the tibia and thickness of its shaft in thyroidec-

TABLE C.

Gigantism (Percent Above Normal) in Skeletal Dimensions of Growth Hormone Injected Intact, Hypophysectomized, and Thyroidectomized Adult Female Rats.

Structure Measured	Intact + Growth Hormone	Hypophysect. + Growth Hormone	Thyroidect. + Growth Hormone	Thyroidect. + Growth Hormone + Thyroxine
Body length, nose to anus	12	+	0	4
Anus to tail-tip	8	15	0	0
Total	10	9	0	+
Cranium, length	10	18	—	4
Thorax, length	16	23	0	5
Vertebrae, length, 5th lumbar	17	+	0	4
9th caudal	13	21	0	0
19th caudal	13	16	0	0
Scapula, length	12	35	+	5
Humerus, length	13	11	0	4
Radius, length	13	15	0	4
Ulna, length	10	15	0	0
Metacarpal III, length	0	0	0	0
Pelvis, length	13	19	0	3
Femur, length	12	23	0	4
Tibia, length	10	17	0	3
Width of head	10	16	+	7
Calcaneus, length	0	0	0	0
Skull, length	7	+	0	0
Thorax, width at 1st rib	11	20	8	7
Width at 10-11th rib	20	×	+	9
Clavicle, length	+	0	0	6
Pelvis, width intercristal	25	27	0	18
Interacetabular	21	29	9	17
Intertuberous	17	18	14	18
Skull, width	15	14	9	15
Cranium, width	8	20	13	10
Vertebra, width 1st lumbar	8	27	12	12
Scapula, width	22	34	12	11
Humerus, height of deltoid tuberosity	17	44	34	35
Pelvis, thickness of ilium	23	41	23	27
Tibia, thickness of shaft	21	32	11	11

TABLE B.

Alterations of Skeletal Dimensions in Adult Female Rats with deficiency or excess of Growth Hormone

Structure Measured	Stunting (% below Normal)		Increase with Growth Hormone (% above Control)		
	Hypophysect. Control	Thyroidect. Control	Hypophysect. + Growth Hormone	Thyroidect. + Growth Hormone	Thyroidect. + Growth Hormone + Thyroxine
Body length, nose to anus	−11	−6	17	5	12
Anus to tail-tip	− 9	−4	26	+	+
Total	−10	−5	22	5	8
Cranium, length	0	0	18	0	7
Thorax, length	0	−7	23	9	14
Vertebrae, length, 5th lumbar	− 7	−7	24	4	11
9th caudal	− 5	−4	28	8	7
19th caudal	− 4	0	22	0	0
Scapula, length	0	0	47	0	6
Humerus, length	−10	−7	23	6	11
Radius, length	− 6	−3	22	4	8
Ulna, length	− 6	−4	25	4	5
Metacarpal III, length	0	0	0	0	0
Pelvis, length	− 5	−4	23	4	8
Femur, length	− 4	−4	28	6	8
Tibia, length	− 5	−3	23	4	6
Width of head	0	0	16	6	10
Calcaneus, length	0	0	0	0	0
Skull, length	− 9	−3	14	0	5
Thorax, width at 1st rib	—	0	25	8	7
Width at 10-11th rib	×	—	×	10	15
Clavicle, length	−17	−6	19	11	13
Pelvis, width intercristal	−14	−5	25	14	26
Interacetabular	−13	−5	49	15	23
Intertuberous	− 9	0	30	16	20
Skull, width	− 5	0	21	11	17
Cranium, width	0	0	21	14	12
Vertebra, width 1st lumbar	0	0	29	16	16
Scapula, width	—	—	43	20	20
Humerus, height of deltoid tuberosity	—	0	65	41	46
Pelvis, thickness of ilium	−13	—	63	29	33
Tibia, thickness of shaft	—	—	37	15	15

tomized growth hormone-treated rats). Changes within the limit of error of comparison (less than 3%) are entered as " 0 ". Changes greater than 3% but not achieving statistically significant levels (usually due to marked variability in response in the experimental group) are entered as " + " or " — ", to indicate their direction. In the hypophysectomized animals, both untreated and growth-hormone treated, the greatest width of the thoracic cage could not be measured from x-rays, and an entry of " X " has been made.

ADDENDUM

Earlier studies with intact and hypophysectomized rats (Evans et al., 1948; Moon et al., 1951) showed that the principal non-endocrine organs responded to chronic high dosage with growth hormone by weight increases proportional to the gain in body weight. An exception existed in the lack of response of the brain and eyeball to growth hormone. The endocrine organs responded negligibly, indicating that contamination with other anterior pituitary hormones was insignificant. Table I gives the visceral weights for growth hormone treated thyroidectomized rats, and shows that the responses were like those previously observed.

Inasmuch as some previous studies (Moon et al., 1950, 1951; Simpson and Evans, 1959) have dealt with the occurrence of tumors during chronic treatment with growth hormone, it should be said here that there were no evidences of growth hormone-induced neoplasia in thyroidectomized rats.

The ovaries of most of the thyroidectomized controls showed some activity (a few growing follicles and some corpora lutea). A variable portion of each ovary, sometimes almost all, had been converted into tubule-like structures as typical of ovaries following thyroidectomy (Evans et al., 1939). The ovarian condition seen in thyroidectomized controls was not appreciably changed by growth hormone administration, with or without thyroxine, although some may have been in follicular development, as suggested by improvement in uterine weight and appearance. In some instances in treated thyroidectomized or normal controls, ovarian or uterine pathology necessitated eliminating organ weights from the mean weight in the group.

ADDENDUM

Table I.

Body and Visceral Weights of Thyroidectomized Rats Injected Chronically with Growth Hormone, and of their Normal and Thyroidectomized Controls.

Experimental Group and Number	Normal Controls (11)		Thyroidectomized Controls (12)		Thyroidectomized + Growth Hormone (6)		Thyroidectomized + Growth Hormone and Thyroxine (6)	
	Weight	% Body Wt.	Weight	% Body Wt.	Weight	% Body Wt.	Weight	% Body Wt.
Body Weight, gm	333 ± 10.4	—	278 ±10.0	—	426 ±18.3	—	474 ±19.6	—
Liver, gm	14.2 ± 1.87	4.2	10.0 ± 0.68	3.6	17.0 ± 0.84	4.0	20.0 ± 1.13	4.2
Spleen, gm	1.1 ± 0.08	0.3	0.5 ± 0.02	0.2	1.2 ± 0.13	0.3	1.4 ± 0.11	0.3
Kidneys (2), gm	2.7 ± 0.06	0.8	1.6 ± 0.05	0.6	2.6 ± 0.14	0.6	3.0 ± 0.09	0.6
Heart, gm	1.0 ± 0.04	0.3	0.7 ± 0.02	0.3	1.2 ± 0.11	0.3	1.3 ± 0.08	0.3
Lungs (2), gm	2.1 ± 0.10	0.6	1.9 ± 0.08	0.7	2.7 ± 0.21	0.6	2.6 ± 0.11	0.5
M. gastrocnemius, gm	1.5 ± 0.03	0.45	1.28 ± 0.07	0.45	1.9 ± 0.11	0.45	2.1 ± 0.13	0.45
Brain, gm	1.9 ± 0.04	0.6	1.74 ± 0.07	0.6	1.8 ± 0.43	0.4	2.0 ± 0.05	0.4
Eyeball (1), mgm	183 ± 3.7	0.0055	180 ± 1.1	0.0065	187 ± 2.6	0.0045	190 ± 5.7	0.0040
Adrenals (2), mgm	75 ± 4.1	—	34 ± 1.5	—	58 ± 5.4	—	78 ± 2.4	—
Thymus, mgm	98 ± 20.5	—	—	—	175 ±24.2	—	109 ±27.0	—
Ovaries (2), mgm	71 ± 6.1	—	54 ± 5.4	—	81 ±13.9	—	117 ±40.6	—
Uterus, mgm	1,198 ±135.8	—	569 ±57.8	—	1,170 ±49.1	—	1,170 ±49.1	—
Pituitary, mgm	16 ± 1.2	—	17 ± 0.9	—	19 ± 0.004	—	19 ± 0.004	—
Specific Gravity of Body, gm/cc	1.03 ± 0.01		1.03 ± 0.01		1.04 ± 0.01		1.03 ± 0.02	
Muscle Nitrogen, mgm/100 mgm	3.47 ± 0.05		3.20 ± 0.06		3.16 ± 0.06		3.39 ± 0.07	
I^{131} Uptake in Paratracheal Region, % total dose	7.34 ± 1.00		0.013 ± 0.002		0.012 ± 0.003		0.008 ± 0.003	

The suprarenal cortex in thyroidectomized controls was narrow, the cell columns disorganized, and the lipid coarser and more irregularly distributed than normal. The narrowing of the cortex was due to a thinner zona fasciculata and reticularis. The zona glomerulosa was not widened. Following growth hormone treatment the suprarenals were larger than in controls.

Thymus weight is not given in Table I for the thyroidectomized controls. Tissue could be found in the thymic region in only four of the twelve animals; the dissectable mass varied from 3 to 172 mgm., the last being nearly all fat.

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LÉGENDE DES PLANCHES I, II ET III.

FIG. 6 to 9.

Photomicrographs of central sagittal sections of proximal epiphyseal cartilage plate of tibia of adult female rats. Hematoxylin and eosin, magnification 62.5.

FIG. 6. Intact control.

FIG. 7. Intact, growth hormone treated.

FIG. 8. Hypophysectomized control.

FIG. 9. Hypophysectomized, growth hormone treated.

FIG. 10 to 12.

Photomicrographs as in Figures 6 to 9.

FIG. 10. Thyroidectomized control.

FIG. 11. Thyroidectomized, growth hormone treated.

FIG. 12. Thyroidectomized, growth hormone treated with thyroxine supplement.

FIG. 13 to 18.

Cross sections of shaft of tibia of adult female rats. Hematoxylin and eosin, magnification 13.

FIG. 13. Intact control.

FIG. 14. Hypophysectomized control.

FIG. 15. Thyroidectomized control.

FIG. 16. Intact, growth hormone treated.

FIG. 17. Hypophysectomized, growth hormone treated.

FIG. 18. Thyroidectomized, growth hormone treated.